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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	09/761,893	HUNG ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston, Ph.D.	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 September 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,4,6,9-20 and 32-38* is/are pending in the application.

4a) Of the above claim(s) *12-20* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,4,6,9-11 and 32-38* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *17 January 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This action is in response to the amendment, filed 9/2/2008, in which claims 1 and 11 were amended, and claims 34-38 were newly added. Claims 1, 4, 6, 9-20 and 32-38 are pending.

Applicant's arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections and objections not reiterated in this action have been withdrawn. **This action is FINAL.**

Election/Restrictions

Applicant elected Group I without traverse in the reply filed on 9/4/2001.

Claims 12-20 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 9/4/2001.

Currently, claims 1, 4, 6, 9-11 and 32-38 are under consideration.

Claim Objections

Claim 1 is objected to because of the following informalities:

1. At line 4, the phrase "said the culturing medium" should be changed to either "said culturing medium" or "the culturing medium" to improve the grammar of the claim. Use of "said the" is redundant.

2. At line 13, the phrase "said the upper plate" should be changed to either "said upper plate" or "the upper plate" to improve the grammar of the claim. Use of "said the" is redundant.

3. At line 14, the phrase "stem cells adhered and cultured" should be changed to "stem cells adhere and are cultured" to improve the grammar of the claim.

4. At line 15, the word "adhered" should be changed to "adhere" to improve the grammar of the claim.

Claims 4, 6, 9-11 and 32-38 depend from claim 1 and thus are objected to for the same reasons applied to claim 1. Appropriate correction is required. This is a new objection, necessitated by the amendment of claim 1 in the reply filed 9/2/2008.

Claims 34-38 are objected to because of the following informalities: the claims do not begin with a capital letter. Appropriate correction is required. This is a new objection, necessitated by the addition of new claims 34-38 in the reply filed 9/2/2008.

Claim 37 is also objected to because of the following informalities: the word "is" should be inserted between the words "plate" and "by" to improve the grammar of the claim. Appropriate correction is required.

Claim 32 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 32 depends from claim 1, which requires "removing non-adherent cells on the upper plate by changing medium." This step is necessarily performed after step (b), because the cells must be seeded into the culture device before non-adherent cells are removed. Thus, claim 32 does not further limit claim 1.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 36-38 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 36 recites the limitation "said recovering" in line 1. There is insufficient antecedent basis for this limitation in the claim. Claim 36 depends from claim 35. Claim 35 does not recite a step of "recovering." Until claim 36, the claims do not provide a step of recovering. It would be remedial to indicate that the method of claim 36 is drawn to the method as claimed in claim 35, "further comprising recovering the confluent mesenchymal stem cells."

Claims 37 and 38 depend from claim 36 and thus are indefinite for the same reason applied to claim 36.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any

evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 4, 6, 9, 11, 32, 33 and 35-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Rieser et al (US Patent No. 6,242,247 B1, cited in a prior action; see the entire reference) and Burkitt et al (Wheater's Functional Histology (1993), page 60, cited in a prior action). This rejection was made in the Office action mailed 8/18/2008 and has been rewritten to address the amendments to the claims in the reply filed 9/2/2008.

Caplan et al teach a method for recovering mesenchymal stem cells from human bone marrow aspirate from iliac crest, femora, tibiae, spine, rib or other medullary spaces, comprising the steps of (i) providing the bone marrow aspirate, which is a cell mixture comprising mesenchymal stem cells and other types of cells, (ii) performing gradient separation with a Percoll gradient, where the upper 25% of the gradient containing mesenchymal stem cells, platelets, and red blood cells is harvested, pelleted and resuspended in DMEM medium, (iii) seeding the cell mixture in DMEM into a device comprising an upper plate comprising a Leukosorb™ filter, which contains pores through which other cells, such as fat cells and red blood cells, pass through, and which retains the mesenchymal stem cells, which adhere to the Leukosorb™ filter, and (iv) recovering the mesenchymal stem cells from the Leukosorb™ filter (upper plate) (e.g., column 8, line 45 to column 9, line 55; column 45, line 41 to column 46, line 34). Caplan et al teach the further enrichment of mesenchymal stem cells from the cell

population recovered from the Leukosorb™ filter specifically by passage over porous hydroxyapatite granules and by monoclonal antibody separation (e.g., column 46, lines 11-61). Further, Caplan et al teach that human mesenchymal stem cells isolated by their methods can be cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 1 g/L of glucose and supplemented with 10% fetal bovine serum to allow the mesenchymal stem cells to grow without differentiation and to allow the direct adherence of only the mesenchymal stem cells to the plastic or glass surface of the culture dish (e.g., column 8, line 20 to column 9, line 45). Further, Caplan et al teach that culturing in DMEM containing 1g/L glucose makes it possible to separate mesenchymal stem cells from other cells such as red and white blood cells, other differentiated mesenchymal stem cells, etc., which are present in bone marrow (e.g., column 8, lines 20-45). Caplan et al teach the removal of the non-adherent matter (i.e., medium and cells that are not adherent) from the culture dish (e.g., column 2, lines 3-19). Thus, Caplan et al generally teach that mesenchymal stem cells can be further enriched by passage over porous hydroxyapatite granules, by monoclonal antibody separation, and by selective adherence in DMEM with glucose and fetal bovine serum. Moreover, Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x $10^3/cm^2$ (e.g.,

column 41). Caplan et al teach that the mesenchymal stem cells can differentiate into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48).

Caplan et al do not specifically teach the method where the cells that pass through the pores of the top plate collect on a lower plate base. Caplan et al do not specifically teach culturing the mesenchymal stem cells on the top plate in 10% fetal bovine serum-supplemented Dulbecco's modified Eagle's medium containing 1 g/L glucose and do not teach removing cells not adhered on the top plate by changing a culture medium.

Rieser et al teach a method comprising the steps of (i) providing bone marrow using a method known in the art, (ii) introducing the bone marrow comprising mesenchymal stem cells to a cell space (1), closing the cell space, and introducing it into the culture medium, which results in the introduction of mesenchymal stem cells above a bone substitute plate (7) (upper plate) and a bottom plate, which is the bottom of the culture dish (e.g., column 5, lines 15-36, column 6, line 56 to column 7, line 3; Figure 1). Rieser et al teach that the cells in the cell space settle on the bone substitute plate (7) due to the effects of gravity (e.g., column 7, lines 24-34). Once the cells have settled on the plate, they adhere and grow (e.g., column 7). Rieser et al teach the subsequent removal of cartilage formed from the cells introduced into the cell space (e.g., paragraph bridging columns 6-7). Rieser et al teach that the bone substitute plate (7) serves two functions: it is a permeable wall for the cell space (1), and it provides a substrate for the adherence of cells (e.g., column 7, lines 7-24). With respect to the porosity of the upper plate (7), Rieser et al teach pores of 1 to 20 μm are suitable, as well as pores between 20 and 50 μm (e.g., column 7, lines 34-54).

Burkitt et al teach that red blood cells are 6.7-7.7 μm in diameter and nucleated cells have a diameter greater than 7.7 μm (page 60).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells in medium into the cell space and culture dish taught by Rieser et al because Caplan et al teach it is within the ordinary skill in the art to use a filter to remove red blood cells from cells of bone marrow aspirate and Riser et al teach the use of a porous filter, where the pore diameter can be modified, in combination with the teachings of Burkitt et al, to allow red blood cells to pass through the pores while the nucleated cells remain on the filter. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use Dulbecco's modified Eagle's medium containing 1 g/L glucose supplemented with 10% fetal bovine serum (DMEM-LG with 10% FBS), taught by Caplan et al, in the culture dish and cell space, because Rieser et al teach culturing the cells in the dish in the presence of medium. Moreover, it would have been obvious to change the medium to allow the continued growth of the cells in an undifferentiated state while removing other non-adherent, non-mesenchymal stem cells as taught by Caplan et al.

One would have been motivated to make such a modification in order to receive the expected benefit of eliminating the extra steps of washing the cells from the filter and performing subsequent purification steps as taught by Caplan et al. The use of the DMEM-LG with 10% FBS and media changes would result in an enriched population of cells. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any

evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Rieser et al (US Patent No. 6,242,247 B1, cited in a prior action; see the entire reference) and Burkitt et al (Wheater's Functional Histology (1993), page 60, cited in a prior action) as applied to claims 1, 4, 6, 9, 11, 32, 33 and 35-38 above, and further in view of Pittenger et al (Science, Vol. 284, pages 143-147, 1999, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 8/18/2008 and has been rewritten to address the amendments to the claims in the reply filed 9/2/2008.

The combined teachings of Caplan et al, Rieser et al, and Burkitt et al et al are described above and applied as before.

Caplan et al, Rieser et al, and Burkitt et al do not specifically teach that the mesenchymal stem cells are CD34-.

Pittenger et al teach the isolation of human mesenchymal cells from bone marrow taken from the iliac crest (e.g., page 143, right column). Pittenger et al teach that the mesenchymal stem cells are CD34- (e.g., paragraph bridging pages 143-144). The mesenchymal stem cells isolated by Pittenger et al are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to specifically use a bone marrow aspirate from human iliac crest, because Caplan et

al and Pittenger et al teach the use of bone marrow from iliac crest to isolate mesenchymal stem cells that are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2). It would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute iliac crest bone marrow for any other type of bone marrow to achieve the predictable result of recovering CD34- mesenchymal stem cells that are also capable of differentiating to adipose, cartilage or bone tissue.

Claims 1, 4, 6, 9, 11, 32 and 35-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Butz et al (US Patent No. 5,272,083; see the entire reference). This rejection was made in the Office action mailed 8/18/2008 and has been rewritten to address the amendments to the claims in the reply filed 9/2/2008.

Caplan et al teach a method for recovering mesenchymal stem cells from human bone marrow aspirate from iliac crest, femora, tibiae, spine, rib or other medullary spaces, comprising the steps of (i) providing the bone marrow aspirate, which is a cell mixture comprising mesenchymal stem cells and other types of cells, (ii) performing gradient separation with a Percoll gradient, where the upper 25% of the gradient containing mesenchymal stem cells, platelets, and red blood cells is harvested, pelleted and resuspended in DMEM medium, (iii) seeding the cell mixture in DMEM into a device comprising an upper plate comprising a Leukosorb™ filter, which contains pores through which other cells, such as fat cells and red blood cells, pass through, and which retains the mesenchymal stem cells, which adhere to the Leukosorb™ filter, and (iv) recovering the mesenchymal stem cells from the Leukosorb™ filter

(upper plate) (e.g., column 8, line 45 to column 9, line 55; column 45, line 41 to column 46, line 34). Caplan et al teach the further enrichment of mesenchymal stem cells from the cell population recovered from the Leukosorb™ filter specifically by passage over porous hydroxyapatite granules and by monoclonal antibody separation (e.g., column 46, lines 11-61). Further, Caplan et al teach that human mesenchymal stem cells isolated by their methods can be cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 1 g/L of glucose and supplemented with 10% fetal bovine serum to allow the mesenchymal stem cells to grow without differentiation and to allow the direct adherence of only the mesenchymal stem cells to the plastic or glass surface of the culture dish (e.g., column 8, line 20 to column 9, line 45). Further, Caplan et al teach that culturing in DMEM containing 1g/L glucose makes it possible to separate mesenchymal stem cells from other cells such as red and white blood cells, other differentiated mesenchymal stem cells, etc., which are present in bone marrow (e.g., column 8, lines 20-45). Caplan et al teach the removal of the non-adherent matter (i.e., medium and cells that are not adherent) from the culture dish (e.g., column 2, lines 3-19). Thus, Caplan et al generally teach that mesenchymal stem cells can be further enriched by passage over porous hydroxyapatite granules, by monoclonal antibody separation, and by selective adherence in DMEM with glucose and fetal bovine serum. Moreover, Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the

recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x $10^3/cm^2$ (e.g., column 41). Caplan et al teach that the mesenchymal stem cells can differentiate into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48).

Caplan et al do not specifically teach the method where the cells that pass through the pores of the top plate collect on a lower plate base. Caplan et al do not specifically teach culturing the mesenchymal stem cells on the top plate in 10% fetal bovine serum-supplemented Dulbecco's modified Eagle's medium containing 1 g/L glucose and do not teach removing cells not adhered on the top plate by changing a culture medium.

Butz et al teach a cell culture device that comprises a cell retention element detachably attached to a hanger (e.g., Abstract; Figure 2). The cell retention element includes a porous membrane growth surface (e.g., column 1, lines 56-57; column 3, lines 50-62). Butz et al teach that the tissue growth membrane may be formed of any material capable of supporting cells substantially isolated from direct contact with the medium in the well, while allowing at least a selected material to pass through and contact the cells (e.g., column 6, lines 17-22). Suitable materials include porous inert film, hydrated gels, or layered combinations such as a gel supported on a screen (e.g., column 6, lines 22-24). Butz et al teach the culture of cells on the membrane and changing the medium in the well containing the cultured cells (e.g., column 6, lines 38-61; column 11, lines 4-25).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells containing mesenchymal stem cells in medium into the retention element of the culture dish taught by Butz et al because Caplan et al

teach it is within the ordinary skill in the art to use a filter to remove fat cells and red blood cells from bone marrow aspirate and Butz et al teach culturing cells in a device comprising a porous filter. It would have been obvious to one of skill in the art to use the Leukosorb filter of Caplan et al, because Butz et al teach the use of any porous inert film, and Caplan et al teach that the Leukosorb filter is suitable for the separation of human mesenchymal stem cells from fat cells and red blood cells. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use Dulbecco's modified Eagle's medium containing 1 g/L glucose supplemented with 10% fetal bovine serum (DMEM-LG with 10% FBS), taught by Caplan et al, in the culture dish of Butz et al, because Butz et al teach culturing the cells in the dish in the presence of medium. Moreover, it would have been obvious to change the medium to allow the continued growth of the cells in an undifferentiated state while removing other non-adherent, non-mesenchymal stem cells, as taught by Caplan et al, because Butz et al teach it is within the skill of the art to change the medium in the culture dish.

One would have been motivated to make such a modification in order to receive the expected benefit of eliminating the extra steps of washing the cells from the filter and performing subsequent purification steps as taught by Caplan et al. The use of the DMEM-LG with 10% FBS and media changes would result in an enriched population of cells. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Butz et al (US Patent No. 5,272,083; see the entire reference) as applied to claims 1, 4, 6, 9, 11, 32 and 35-38 above, and further in view of Pittenger et al (Science, Vol. 284, pages 143-147, 1999, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 8/18/2008 and has been rewritten to address the amendments to the claims in the reply filed 9/2/2008.

The combined teachings of Caplan et al and Butz et al are described above and applied as before.

Caplan et al and Butz et al do not specifically teach that the mesenchymal stem cells are CD34-.

Pittenger et al teach the isolation of human mesenchymal cells from bone marrow taken from the iliac crest (e.g., page 143, right column). Pittenger et al teach that the mesenchymal stem cells are CD34- (e.g., paragraph bridging pages 143-144). The mesenchymal stem cells isolated by Pittenger et al are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to specifically use a bone marrow aspirate from human iliac crest, because Caplan et al and Pittenger et al teach the use of bone marrow from iliac crest to isolate mesenchymal stem cells that are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2). It would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute iliac crest bone marrow for any other type of bone marrow to achieve the predictable

result of recovering CD34- mesenchymal stem cells that are also capable of differentiating to adipose, cartilage or bone tissue.

Claim 33 is rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Butz et al (US Patent No. 5,272,083; see the entire reference) as applied to claims 1, 4, 6, 9, 11, 32 and 35-38 above, and further in view of Pall (US Patent No. 4,923,620; see the entire reference). This rejection was made in the Office action mailed 8/18/2008 and has been rewritten to address the amendments to the claims in the reply filed 9/2/2008.

The combined teachings of Caplan et al and Butz et al are described above and applied as before.

Caplan et al and Butz et al do not specifically teach the pore size of the Leukosorb filter.

Pall teaches filters to remove leukocytes from whole blood, where the pore diameter is from about 4 to about 8 micrometers, from about 4 to about 5.5 micrometers, or from about 6 to about 8 micrometers (e.g., column 9, lines 37-57).

Because Caplan et al and Pall teach filters to remove leukocytes, and Caplan teaches that this type of filter can be used to isolate mesenchymal stem cells, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use a pore size of about 4 to about 8 micrometers in the method of Caplan et al and Butz et al to achieve the predictable result of separating mesenchymal stem cells from red blood cells and fat cells.

Claims 1, 4, 6, 9, 11 and 32-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Prockop et al (US Patent No. 7,374,937 B1, effective date March 14, 2000; see the entire reference) and Mastui et al (US Patent No. 4,871,674; see the entire reference). This is a new rejection, necessitated by the addition of new claim 34.

Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by replacing the original complete medium with fresh complete medium (e.g., column 11, line 63 to column 12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x

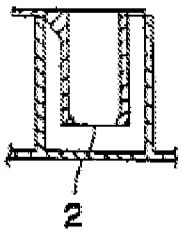
$10^3/cm^2$ (e.g., column 41). Caplan et al teach that the mesenchymal stem cells can differentiate into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48). Moreover, Caplan et al teach that a porous filter can be used to remove red blood cells from the mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34).

Caplan et al do not teach the method of isolating human mesenchymal stem cells where the mixed population of cells in medium is seeded into a culture device comprising an upper plate with pores and a lower plate base, where small cells pass through the pores in the upper plate and adhere to the lower plate.

Prockop et al teach that RS cells can be separated from non-RS mesenchymal stem cells by ultrafiltration. Prockop et al teach that smaller RS cells will pass through an ultrafiltration membrane having appropriately sized pores, and such a membrane is a Millipore brand 10 micrometer isopore polycarbonate (plastic) membrane (e.g., column 39, line 60 to column 40, line 42).

Matsui et al teach culturing cells in a cell culture device comprising a cell culture insert comprising a membrane filter (2) on the bottom of the culture cell, which is composed of polycarbonate (e.g., column 2, lines 41-55; column 3, lines 5-18). The culture device is shown in Figure 8, which is reproduced below:

Fig. 8



It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into the culture dish taught by Matsui et al because Caplan et al teach it is within the ordinary skill in the art to use a filter to remove fat cells and red blood cells from cells of bone marrow, Prockop et al teach the collection of mesenchymal stem cells on a filter of polycarbonate containing 10 micrometer pores, and Matsui et al teach culturing cells in a device comprising a polycarbonate filter.

One would have been motivated to make such a modification in order to receive the expected benefit of providing an enriched population of mesenchymal stem cells without having to perform the extra steps of using a separate filter as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Matsui et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 4, 6, 9, 11 and 32-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Burkitt et al (Wheater's Functional Histology (1993), page 60, cited in a prior action) and Mussi et al (US Patent No. 5,409,829; see the entire reference). This is a new rejection, necessitated by the addition of new claim 34.

Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by replacing the original complete medium with fresh complete medium (e.g., column 11, line 63 to column 12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x

$10^3/cm^2$ (e.g., column 41). Caplan et al teach that the mesenchymal stem cells can differentiate into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48). Moreover, Caplan et al teach that a porous filter can be used to remove red blood cells from the mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34).

Caplan et al do not teach the method of isolating human mesenchymal stem cells where the mixed population of cells in medium is seeded into a culture device comprising an upper plate with pores and a lower plate base, where small cells pass through the pores in the upper plate and adhere to the lower plate.

Burkitt et al teach that red blood cells are 6.7-7.7 μm in diameter and nucleated cells have a diameter greater than 7.7 μm (page 60).

Mussi et al teach the introduction of a mixture of cells to be grown into a culture chamber in a suitable growth medium (e.g., column 2, lines 46-50). Mussi et al teach that the cells are grown in a culture insert contained within a well, where the insert is suspended in the well (e.g., paragraph bridging columns 3-4; Figure 4). The culture insert contains a membrane (20), which may be formed from a polymeric material such as polyethylene terephthalate, polycarbonate, and the like with open pores throughout (e.g., column 3, lines 50-53). Mussi et al teach that the pores are between about 0.2 to about 10 microns in diameter (e.g., column 3, lines 53-57).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells in medium into the culture insert of the culture device of Mussi et al because Caplan et al teach it is

within the ordinary skill in the art to use a filter to remove red blood cells from cells of bone marrow aspirate and Mussi et al teach the use of a porous polycarbonate filter membrane, where the pore diameter can be about 0.2 to about 10 microns in diameter, and Burkitt et al teach that red blood cells are the size which would pass through the filter of Mussi et al while nucleated mesenchymal stem cells of Caplan et al would be retained on top.

One would have been motivated to make such a modification in order to provide an enriched population of mesenchymal stem cells without the extra steps of using a column containing a filter as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Mussi et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 4, 6, 9, 11, 32 and 34-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Guirguis (US Patent No. 5,077,012; see the entire reference) and Mastui et al (US Patent No. 4,871,674; see the entire reference). This is a new rejection, necessitated by the addition of new claim 34.

Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of

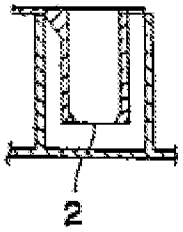
Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by replacing the original complete medium with fresh complete medium (e.g., column 11, line 63 to column 12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x $10^3/cm^2$ (e.g., column 41). Caplan et al teach that the mesenchymal stem cells can differentiate into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48). Moreover, Caplan et al teach that a porous filter can be used to remove red blood cells from the mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34).

Caplan et al do not teach the method of isolating human mesenchymal stem cells where the mixed population of cells in medium is seeded into a culture device comprising an upper plate with pores and a lower plate base, where small cells pass through the pores in the upper plate and adhere to the lower plate.

Guirguis teaches the removal of red blood cells from a body fluid using a membrane with a smooth flat surface which is ideal for the collection of atypical cells from all types of body fluids (e.g., column 3, lines 37-45; column 4). Guirguis teaches that the advantage of using a polycarbonate membrane is the minimum clogging by red blood cells and protein, well preserved cellular morphology with a high recovery rate, and excellent surface capture due to the pore structure and porosity (e.g., column 4, lines 43-64).

Matsui et al teach culturing cells in a cell culture device comprising a cell culture insert comprising a membrane filter (2) on the bottom of the culture cell, which is composed of polycarbonate (e.g., column 2, lines 41-55; column 3, lines 5-18). The culture device is shown in Figure 8, which is reproduced below:

Fig. 8



It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into the culture dish taught by Matsui et al because Caplan et al teach it is within the ordinary skill in the art to use a filter to remove fat cells and red blood cells from cells of bone marrow, Guirguis teaches the use of a polycarbonate membrane for the removal of red blood

cells from a body fluid, and Matsui et al teach culturing cells in a device comprising a polycarbonate filter.

One would have been motivated to make such a modification in order to receive the expected benefit of providing an enriched population of mesenchymal stem cells without having to perform the extra steps of using a separate filter as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Matsui et al. Further, one would have been motivated to use the polycarbonate (plastic) filter in place of the Leukosorb filter taught by Caplan et al, because Caplan et al teach that mesenchymal stem cells become selectively attached to plastic in DMEM containing 10% FBS and 1 g/L of glucose or complete medium, and Guirguis teaches that the advantage of using a polycarbonate membrane is the minimum clogging by red blood cells and protein, well preserved cellular morphology with a high recovery rate, and excellent surface capture due to the pore structure and porosity of the polycarbonate. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Response to Arguments - 35 USC § 103

With respect to the rejection of claims 1, 4, 6, 9, 11, 32, 33 and 35-38 under 35 U.S.C. 103(a) as being unpatentable over Caplan et al in view of Rieser et al and Burkitt et al; and the rejection of claim 10 under 35 U.S.C. 103(a) as being unpatentable over Caplan et al in view of Rieser et al and Burkitt et al and further in view of Pittenger et al, Applicant's arguments filed 9/2/2008 have been fully considered but they are not persuasive.

The response asserts that Caplan et al does not provide the bone marrow aspirate with a culture medium that facilitates mesenchymal stem cell growth. This argument is not found persuasive. The claims require "providing a cell mixture comprising mesenchymal stem cells and other cells in a culture medium," where the culture medium contains factors that stimulate mesenchymal stem cell growth without differentiation. Caplan et al teach providing a cell mixture from bone marrow aspirate and combining the cell mixture with DMEM or complete medium, which contains factors that stimulates mesenchymal stem cell growth without differentiation (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34).

The response asserts that the instant application provides a method for isolating, culturing and expanding mesenchymal stem cells to confluence at the same time, and, therefore, it would not be obvious to one of ordinary skill in the art to modify the method of isolating mesenchymal stem cells of Caplan et al. This argument is not found persuasive. Caplan et al teach that mesenchymal stem cells can be isolated, cultured and expanded to confluence (e.g., column 11, line 63 to column 12, line 41; column 19, line 45 to column 20, line 14). Caplan et al teach that a filter can be used to remove red blood cells from a mixed population of cells comprising stem cells and mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34). The rejection of record is based upon the combined teachings of Caplan et al, Rieser et al and Burkitt et al. The ordinary skilled artisan, upon reading the teachings of these three references, would recognize that the filter material could be placed in a culture vessel, and red blood cells could be removed by gravity flow through the filter. Given the teachings of Caplan et al, it would have

been obvious to one of skill in the art to culture the cells on the filter material in the context of a culture dish taught by Rieser et al.

The response asserts that because Caplan et al do not provide the bone marrow aspirate with the culture medium, modification of Caplan et al with Rieser et al does not result in the claimed invention. This argument is not found persuasive. The claims require "providing a cell mixture comprising mesenchymal stem cells and other cells in a culture medium," where the culture medium contains factors that stimulate mesenchymal stem cell growth without differentiation. Caplan et al teach providing a cell mixture from bone marrow aspirate and combining the cell mixture with DMEM or complete medium, which contains factors that stimulates mesenchymal stem cell growth without differentiation (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34).

The response asserts that the steps of removing non-adherent cells are not identified in the prior art. This argument is not persuasive, because Caplan et al teach the steps of removing non-adherent cells. Specifically, Caplan et al teach that human mesenchymal stem cells isolated by their methods can be cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 1 g/L of glucose and supplemented with 10% fetal bovine serum to allow the mesenchymal stem cells to grow without differentiation and to allow the direct adherence of only the mesenchymal stem cells to the plastic or glass surface of the culture dish (e.g., column 8, line 20 to column 9, line 45).

The response asserts that the pores of Rieser et al are pores that function for cell growth as areas of surface roughness but cannot be used to separate cells through the pores. This argument is not found persuasive. Rieser et al teach that the bone substitute plate (7) contains

pores that form continuous canals (open porosity) and these pores are 2 to 20 μm (e.g., column 7, line 55 to column 8, line 16). The 300 to 700 μm pores refer to the surface pores (surface roughness) (e.g., column 8, lines 1-16) and not the open porosity (disclosed as 1 to 20 μm at column 12, line 35). The size of the open pores taught by Rieser et al is consistent with the size of the pores required to filter red blood cells from the sample. There is no evidence of record that indicates that the red blood cells would not pass through these pores and Applicant has not provided a side-by-side comparison of the efficacy of Rieser's culture dish and the culture dish of the present specification to demonstrate unexpected results as they relate to the claimed invention.

The response asserts that since the bone substitute plate of Rieser et al is used for implantation, it would not be obvious to remove non-adherent cells by changing medium to isolate the mesenchymal stem cells from the upper plate. Further, the response asserts that one would not think to remove non-adherent cells from a plate of implantation. Moreover, the response asserts that it would be difficult to remove cells due to the surface roughness. These arguments are not found persuasive. Caplan et al teach that human mesenchymal stem cells isolated by their methods can be cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 1 g/L of glucose and supplemented with 10% fetal bovine serum to allow the mesenchymal stem cells to grow without differentiation and to allow the direct adherence of only the mesenchymal stem cells to the plastic or glass surface of the culture dish (e.g., column 8, line 20 to column 9, line 45). Since the medium used prevents adherence of cells other than mesenchymal stem cells, it would have been within the skill of the art to change the medium, as taught by Caplan et al, to remove non-adherent cells. Since the cells do not adhere, they would

be easily removed. Further, Rieser et al teach the use of any bone substitute material known in the art (e.g., column 8, lines 21-25). Rieser et al teach the production of flexible plates from collagen I, from collagen II and hydroxyapatite or from poly-lactic acid, and the construction of rigid plates from tricalcium-phosphate, from hydroxyapatite or from other inorganic bone substitute materials (e.g., column 8, lines 21-25). Caplan et al teach the removal of cells from culture dishes using a releasing agent such as trypsin with EDTA or a chelating agent such as EGTA (e.g., column 12, lines 26-41). One would expect the trypsin treatment of Caplan et al to result in the removal of cells from a bone substitute plate taught by Rieser et al.

The response asserts that the function, way and result of the upper plate of the present application are different from Caplan et al and Rieser et al for the following reasons:

	This application	Caplan et al + Rieser et al
function	The pores of the upper plate is to separate the mesenchymal stem cells from other cells through the pores	growing in the cell space grows into pores or surface roughness of the plate
way	the upper plate made of the mesenchymal stem cell adhering material, said plastic	bone substitute plate is rigid. , plastically deferrable, biologically degradable or not degradable.
result	isolate mesenchymal stem cells from the bone marrow aspirate	to implant the bone substitute plate

These arguments are not persuasive. As discussed above, the plate of Rieser et al contains open pores and not just surface roughness as asserted by Applicant. Further, the instant claims do not limit the plate material to plastic. The rejected claims encompass the use of any material to which mesenchymal stem cells can adhere. Applicant has not provided evidence that mesenchymal stem cells do not adhere to the materials taught by Rieser et al. The response asserts that the combination of Caplan et al and Rieser et al results in implantation of the bone

substitute plate. This is not found persuasive. Caplan et al teach the isolation of mesenchymal stem cells using medium that allows the proliferation of stem cells without differentiation. The use of the bone substitute plate as a filter to isolate mesenchymal stem cells does not necessarily result in implantation of the bone substitute plate.

The response asserts that Rieser suggests that "it is not necessary to isolate specific cell types from donor tissue, i.e. mixtures of different cells as usually contained in such tissues can be brought into such a space." (column 5 lines 26-28). This statement is completely consistent with the claims, which require the introduction of a mixture of cells into a culture device containing a porous plate.

The response asserts that Rieser et al discredit the filter material of US Patent No. 5,326,357 and thus one would not use the material of Rieser et al. This argument is not found persuasive, because the teachings of US Patent No. 5,326,357 are not relied upon in the instant rejection and do not speak to the combination of references used. The rejection is not based upon the use of the filter material of US Patent No. 5,326,357.

The response asserts that the pores of Rieser et al are not pores that function for cell growth as areas of surface roughness but cannot be used to separate cells through the pores. This argument is not found persuasive. Rieser et al teach that the bone substitute plate (7) contains pores that form continuous canals (open porosity) and these pores are 2 to 20 μm (e.g., column 7, line 55 to column 8, line 16). The 300 to 700 μm pores refer to the surface pores (surface roughness) (e.g., column 8, lines 1-16) and not the open porosity (disclosed as 1 to 20 μm at column 12, line 35). The size of the pores taught by Rieser et al is consistent with the size of the pores required to filter red blood cells from the sample. There is no evidence of record that

indicates that the red blood cells would not pass through these pores and Applicant has not provided a side-by-side comparison of the efficacy of Rieser's culture dish and the culture dish of the present specification to demonstrate unexpected results as they relate to the claimed invention.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

With respect to the rejection of claims 1, 4, 6, 9, 11, 32 and 35-38 under 35 U.S.C. 103(a) as being unpatentable over Caplan et al in view of Butz et al; the rejection of claim 10 under 35 U.S.C. 103(a) as being unpatentable over Caplan et al in view of Butz et al and further in view of Pittenger et al; and the rejection of claim 33 under 35 U.S.C. 103(a) as being unpatentable over Caplan et al in view of Butz et al and further in view of Pall, Applicant's arguments filed 9/2/2008 have been fully considered but they are not persuasive.

The response asserts that Caplan et al does not provide the bone marrow aspirate with a culture medium that facilitates mesenchymal stem cell growth. This argument is not found persuasive. The claims require "providing a cell mixture comprising mesenchymal stem cells and other cells in a culture medium," where the culture medium contains factors that stimulate mesenchymal stem cell growth without differentiation. Caplan et al teach providing a cell mixture from bone marrow aspirate and combining the cell mixture with DMEM or complete medium, which contains factors that stimulates mesenchymal stem cell growth without differentiation (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34).

The response asserts that the instant application provides a method for isolating, culturing and expanding mesenchymal stem cells to confluence at the same time, and, therefore, it would not be obvious to one of ordinary skill in the art to modify the method of isolating mesenchymal stem cells of Caplan et al. This argument is not found persuasive. Caplan et al teach that mesenchymal stem cells can be isolated, cultured and expanded to confluence (e.g., column 11, line 63 to column 12, line 41; column 19, line 45 to column 20, line 14). Caplan et al teach that a filter can be used to remove red blood cells from a mixed population of cells comprising stem cells and mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34). The rejection of record is based upon the combined teachings of Caplan et al, Rieser et al and Burkitt et al. The ordinary skilled artisan, upon reading the teachings of these three references, would recognize that the filter material could be placed in a culture vessel, and red blood cells could be removed by gravity flow through the filter. Given the teachings of Caplan et al, it would have been obvious to one of skill in the art to culture the cells on the filter material in the context of a culture dish taught by Rieser et al.

The response asserts that because Caplan et al do not provide the bone marrow aspirate with the culture medium, modification of Caplan et al with Rieser et al does not result in the claimed invention. This argument is not found persuasive. The claims require "providing a cell mixture comprising mesenchymal stem cells and other cells in a culture medium," where the culture medium contains factors that stimulate mesenchymal stem cell growth without differentiation. Caplan et al teach providing a cell mixture from bone marrow aspirate and combining the cell mixture with DMEM or complete medium, which contains factors that

stimulates mesenchymal stem cell growth without differentiation (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34).

The response asserts that the steps of removing non-adherent cells are not identified in the prior art. This argument is not persuasive, because Caplan et al teach the steps of removing non-adherent cells. Specifically, Caplan et al teach that human mesenchymal stem cells isolated by their methods can be cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 1 g/L of glucose and supplemented with 10% fetal bovine serum to allow the mesenchymal stem cells to grow without differentiation and to allow the direct adherence of only the mesenchymal stem cells to the plastic or glass surface of the culture dish (e.g., column 8, line 20 to column 9, line 45).

The response asserts that the function, way and result of the upper plate in the present application are different from the combination of Caplan et al and Butz et al for the following reasons:

	This application	Caplan et al + Butz et al
function	The pores of the upper plate is to separate the mesenchymal stem cells from other cells through the pores	The cell retention element includes a porous membrane growth surface.(column 1 lines 56-57)
way	the upper plate made of the mesenchymal stem cell adhering material, said plastic	It is formed of two separate pieces, rather than as a unitary piece as in the transwell. (column 1 lines 44-45) The retention element 10 has a hollow, cylindrical side wall defining an annular bottom surface. Attached to the annual surface is a tissue growth member. The tissue

		growth membrane may be formed of any material capable of supporting cells or tissue substantially isolated from direct contact with medium in the wall, while allowing at least selected material to pass through and contact the cells. Such materials include porous iner film,...(column 6 lines 14-22)
result	isolate mesenchymal stem cells from the bone marrow aspirate	A tissue or cell growth device

These arguments are not found persuasive. The response asserts that the pores of the plate of the instant application separate cells. The Leukosorb filter of Caplan et al serves the same function (e.g., Caplan et al, column 46, lines 11-34). The response asserts that the plate of the instant invention is made of plastic; however, the rejected claims are not limited to an upper plate of plastic. The Leukosorb filter of Caplan et al falls within the scope of what is claimed. The result of the combined teachings of the prior art is the growth of mesenchymal stem cells, which are isolated from bone marrow aspirate as taught by Caplan et al (e.g., column 45, line 45 to column 46, line 34). The combination of known elements taught by Caplan et al and Butz et al is a predictable use of prior art elements according to their established functions, and the particular combination falls within the scope of what is claimed.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached at 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Jennifer Dunston, Ph.D.
Examiner
Art Unit 1636

/JD/

/Celine X Qian /
Primary Examiner, Art Unit 1636